SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1 Purification of cerebellar stem cells and assessment of neurosphere forming-efficiency. To enrich for stem cells, cells from the cerebellum of Math1-GFP mice (cerebellar suspension) were stained with antibodies specific for Prominin and for neuronal and glial lineage markers (O4, TAPA-1 and PSA-NCAM). Cells were then FACS-sorted into GFP+ (GCP) and GFP– (non-GCP) fractions, representing 90% and 10% of the starting population respectively. GFP– cells were further fractionated into Prominin– cells (7% of the cerebellar suspension), Prominin+Lineage+ cells (2.8%) and Prominin+Lineage– cells (0.2%). Each of these fractions was cultured at clonal density in bFGF and EGF for 10 days, and then the neurosphere forming efficiency (neurospheres/cells plated, shown in orange boxes) was determined. The starting cerebellar suspension formed one neurosphere for every 1000 cells plated. GCPs (Math1-GFP+ cells) could not form neurospheres when cultured at clonal density in the presence of bFGF + EGF or Sonic hedgehog; in contrast, Math1-GFP– cells showed a 10-fold enrichment in neurosphere-forming ability (1 neurosphere/100 cells) compared to the starting cell suspension. Prominin– cells survived poorly and did not form neurospheres at clonal density. Prominin+Lineage+ cells formed few free-floating neurospheres (<1 neurosphere/100 cells), but often gave rise to adherent colonies that underwent neuronal differentiation. Prominin+Lineage– cells formed one neurosphere for every 30 cells plated.

Supplementary Figure 2. Self-renewal of cerebellar stem cells. (a) Primary and secondary neurospheres. FACS-sorted Prominin+Lin– cells were cultured at clonal density in media containing bFGF and EGF. After 10 days, primary (1°) neurospheres were photographed at 20X magnification. Primary neurospheres were then dissociated and replated under the conditions described above. Secondary (2°) neurospheres, photographed at 10 days, resembled primary neurospheres in morphology and frequency. Neurospheres could be propagated in this manner for up to 10 weeks *in vitro*. **(b) Efficiency of neurosphere formation at clonal density and after repeated passage in culture.** The efficiency of neurosphere formation was similar whether Prominin+Lineage– cells were cultured at a density of 1 cell per well or at a density of 1 cell per mm^2 (clonal density). Moreover, neurosphere-forming efficiency was maintained in successive passages of neurospheres generated at clonal density. In all cases, approximately 1 neurosphere was generated for every 30 cells plated.